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Evaluation of Site-Specific Criteria for Copper and Zinc: An Integration of Metal Addition Toxicity, Effluent and Receiving Water Toxicity, and Ecological Survey Data.

by

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16. ABSTRACT

Comparative laboratory toxicity tests using daphnids Ceriodaphnia dubia and fathead minnows Pimephales promelas were conducted to establish and evaluate relationships between the toxicity of domestic and industrial effluents containing copper and zinc, toxicity of the effluents in Naugatuck River, Connecticut receiving water, toxicity of each metal added to the receiving water and a reference water, and receiving water ecological survey data. The relationships were used to determine if site-specific water quality criteria for copper and zinc derived according to U.S. Environmental Protection Agency (U.S. EPA) guidelines were protective of aquatic life under complexed ambient conditio s caused by point source effluents.

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INTRODUCTION

The physical and/or chemical characteristics of water in a natural system may alter the biological availability and/or toxicity of materials such as copper and zinc. Guidelines for deriving site-specific water quality criteria for the protection of aquatic life and its uses [1,2] which take these factors into account (hereafter referred to as the site-specific guidelines) have been provided by the U.S. Environmental Protection Agency (U.S. EPA). One guideline approach is to simply test a prescribed number of resident species in site water to meet minimum data requirements from which a site criterion is calculated. Another approach is to test sensitive "indicator or surrogate species" from the same population in both clean reference water, hereafter referred to as laboratory water, and site water at the same time under similar conditions except for water characteristics. The ratio of the site water toxicity value/lab water toxicity value is used to modify the national criteria value to a site-specific value. Both of these criteria derivation approaches are based on the assumptions: (1) that differences in the toxicity values of a specific material determined in laboratory water and site water may be attributed to chemical (e.g., complexing ligands) and/or physical (e.g., adsorption) factors that alter the biological availability and/or toxicity of a material and (2) that selected test species directly integrate differences in the biological availability and/or toxicity of the material and provide a direct measure of the capacity of a site water to increase or decrease toxicity values relative to values obtained in laboratory water.

Single chemical criteria address effects of pollutants on aquatic life in the absence of other pollutants in the water column, a condition which seldom occurs. A chemical of interest is usually one component of many

components in an effluent which may affect the chemicals biological availability and/or toxicity. The objective of this research project was to determine if site-specific water quality criteria derived for copper and zinc using the indicator species procedure were protective of aquatic life under complexed ambient conditions caused by point source effluents.

The research objective was approached by conducting comparative aquatic toxicity tests to establish toxicity relationships for the metals of interest between a reference water, a relatively unpolluted upstream river water, downstream waters containing effluents and expected to contain the metals of interest in excess of national water quality criteria, and ecological survey data.

This study on derivation and effectiveness of site-specific criteria was integrated with a larger receiving and effluent water study of Mount et al. [3] designed to investigate the use of laboratory effluent toxicity tests to predict ambient stream toxicity impacts at a multiple discharge site on a medium-size river system. The study area extended from Torrington to Ansonia, Connecticut and encompassed 50 kilometers (30 miles) of the Naugatuck River and included both domestic and industrial waste discharges. The industries are mostly small metal refinishing facilities that discharge wastes into tributaries.

MATERIALS AND METHODS

River, tributary, and effluent sampling

Mount et al. [3] established twelve Naugatuck River sampling stations above and below the Torrington (TRN), Thomaston (THM), Waterbury (WTB), and Naugatuck (NGT) sewage treatment plant (STP) discharges and Gulf Stream (GS), Steele Brook (SB), Great Brook (GB), and Mad River (M) tributaries to the river (Figure 1). The STPs and tributaries which were considered point source effluents were also sampled. Sampling station 1 was located on the west branch of the Naugatuck (N) upstream from all point source discharges and was used as the control and dilution water source. Most water samples collected were 24 hour composite samples with sampling done every 15 minutes. Station 1 samples were daily grab samples. Daily grab samples were taken from station 3 on 23, 24 and 27 August 1983, station 4 on 28 August 1983, station 9 on 24 August 1983, and station 10 on 23 August 1983. Stations 6 and 7 were composites of four grab samples of equal volume taken at six hour intervals over a 24 hour period. On the day of collection subsamples of test waters were used in toxicity tests and the rest stored at 6 C for later use. The tests were conducted in a mobile laboratory located on site. Subsamples were also transported to an off-site laboratory for use in effluent dilution toxicity tests.

Toxicity testing

Water sampling commenced on 22 August 1983 and receiving water toxicity tests of up to seven days duration started the next day by Mount et al. [3]. Daphnids Ceriodaphnia dubia and larval fathead minnows Pimephales promelas from laboratory cultures were used as the test species. For comparative purpose (this study), Ceriodaphnia dubia were exposed in seven day tests to copper and zinc additions to station 1 water collected on 26 August 1984.

These two test species and a resident daphnid Scaphrolebaris sp. were also exposed to copper and zinc added to Lake Superior water (reference) and water samples collected on 31 August 1983 from river stations 1, 4A, 5, 6, 7 in acute toxicity tests. These stations were selected for testing based on the completed and ongoing receiving water toxicity tests. Additional acute toxicity tests were conducted with water collected from station 1 on 5 September 1983 and transported within 48 hr to the U.S. EPA Environmental Research Laboratory at Duluth, Minnesota and stored at 6 C prior to use.

The daphnids used in the seven day tests were placed one animal to each of ten 30-ml plastic cups for each effluent concentration, receiving water sample, or metal addition sample tested. Fifteen ml of the test water was placed in each cup and a daphnid, less than 4 hr old, was added. One drop of water containing 250 µg yeast was added daily. Each daphnid was moved on day 2 and 4 of the test to a new cup containing 15 ml volume of water obtained from the stored sample. When young were present, they were counted and discarded. Toxicity endpoints measured were effects on survival and young production of originally exposed females. For additional details of the test procedure used see Mount and Norberg [4].

The laboratory stock of Ceriodaphnia dubia used were from the U.S. EPA Environmental Research Laboratory, Duluth, Minnesota, culture unit which is maintained in Lake Superior water. Gravid females had been placed in water from station 1 on 22 October 1983 from which young were drawn for testing. Some females were also maintained in Lake Superior water from which young were drawn for use in tests in this water.

Adult Scaphrolebaris sp. were collected from station 5 and maintained in station 1 water for approximately 1 or 2 days prior to use in the acute toxicity tests with copper. Collections were made from shallow areas on the

edge of the river where there was no apparent current. Algae mats containing the daphnids were scooped into plastic pails and transferred to the mobile laboratory. Like animals were selected for testing by their behavior.

The on-site copper and zinc acute toxicity tests were conducted using the laboratory stock daphnids (<4-hr-old), site collected daphnids (adults), and fathead minnows (<24-hr-old) from the U.S. EPA Environmental Research Laboratory, Duluth culture unit. Ten or twenty animals were tested at each of 5 concentrations and a control. A one to three dilution factor was used. Five daphnids were placed into duplicate test containers at each treatment. The daphnids tested in water collected on 31 September 1983 were placed in 30 ml plastic cups containing 15 ml of water and exposed for 48 hr under static water conditions. Glass beakers containing 30 ml of water were used in the off-site tests conducted with water collected on 5 September 1983. A one to two dilution factor was used. The daphnids used in these tests were <24 hr old. Ten fathead minnows were placed in 1,000 ml polyethylene (PE) test chambers containing 700 ml of water collected on 31 August 1983 and exposed for 96 hr under static water conditions. Duplicate test chambers were used for each copper and zinc concentration and type of test water. Also, an additional test chamber containing the test water but no organisms, was set up for each copper concentration and type of test water, so that electrode determinations of free copper (Cu^{+2}) activities could be made. Duplicate 500 ml glass beakers containing 180 ml of water and a one to two dilution factor were used in the fathead minnow (<24 hr old) tests of water collected on 5 September 1983.

Physical and chemical

Test temperature in the mobile laboratory ranged between 22 and 28 C over the on-site test period. The recorded temperature changes were gradual

and associated with diurnal ambient temperature fluctuations and should not have been a problem. Test temperatures in the toxicity tests using water collected on 5 September 1983 ranged between 24 and 25 C. All water samples used in testing were stored in 20 liter PE containers. Subsamples were analyzed for pH, alkalinity, total hardness, turbidity, conductivity, nitrite and chlorine content within 12 hours of subsampling. Samples were kept in an air-conditioned laboratory to prevent overheating. Conductivity, nitrite, and chlorine measurements were not made every day. Dissolved oxygen concentrations were at or near air-saturation concentrations at the beginning of each test or after a water change. Dissolved oxygen concentrations of water in selected treatments from all tests were measured at the end of a test or just before a water change in order to estimate minimum occurring concentrations. U.S. EPA approved procedures [5] were used for all analysis. Subsamples were preserved for analysis of ammonia, nitrate, non-filterable residue and total organic carbon (TOC) following U.S. EPA appoved procedures [5]. Ammonia and nitrate samples were taken daily by filling 250 ml PE bottles, adding 400 µl of concentrated sulfuric acid and placing the samples in coolers containing ice. Nonfilterable residue samples (180 ml) and total organic carbon samples (250 ml) were placed on ice and frozen upon arrival at the University of Wisconsin-Superior laboratory.

Subsamples of all test waters were preserved for later total acid exchangeable (non-filtered) and dissolved (filtered, 0.45 µ) metal analysis. A 25 ml portion of subsample, both non-filtered and filtered, were stored in PE or polypropylene (PP) bottles. A set of subsamples collected on 29 August 1983 were preserved for later total recoverable metal analyses [5] for use in comparison to acid exchangeable (total recoverable procedure minus the digestive process) metal values.

U.S. EPA approved procedures [5] were used for sampling, flame and flameless atomic absorption spectrophotometry (AA) and inductively coupled plasma-atomic absorption emissions spectrometer (ICPAES) analysis of water samples for metals. For quality control assessment, spikes and reference samples were developed at the time of AA analysis. Percentage recovery and standard deviations of spiked samples for copper (N=27) and zinc (N=12) were 101.2 $\pm$ 3.7 and 102.5 $\pm$ 6.4, respectively.

Generally, copper AA measurements were made on all water samples collected. The samples analyzed by AA for zinc were those collected on the 23, 24, 26, 29 and 31 of August 1983. ICPAES analysis was performed on the 26 and 31 August samples and a reference water sample.

The free copper (Cu^{+2}) activities were determined in water from the test chambers without organisms within the first 24 hours of the beginning of each fathead minnow acute toxicity test. Additional free copper activities were also determined in water from the exposure chambers with organisms after the termination of the tests. However, due to time limitations, free copper determinations after the termination of the tests were performed only in water from exposure chambers bracketing the LC50 point.

The free copper determinations were performed as follows. Water from a given exposure chamber was poured into a 500 ml polyethylene bottle to the top of the bottle, and then closed to the atmosphere with a screw polyethylene top into which a cupric ion selective electrode, double junction reference electrode and pH electrode had been previously tightly fitted. The sample was closed to the atmosphere with minimal head space, to prevent CO_2 exchange and the associated pH changes from occurring during the stirring required for the free copper determinations. The sample was then stirred

with a Teflon bar, and mV readings were recorded until they leveled off to changes of no more than 0.1 mV in a 5 minute period. For additional procedural details see Nelson et al. [6].

The copper and zinc exposure stock solutions were prepared using Fisher reagent grade copper-chloride and zinc-chloride dissolved in deionized water.

Statistical procedures

The trimmed Spearman-Karber Method [7] was used for estimating median lethal concentration (LC50s) or effect concentrations (EC50s). The EC50 values calculated were based on the total number of dead fish plus fish with impaired mobility observed at each test concentration. One way analysis of variance and Dunnett's procedure [8] for comparing all treatments with a control (P=0.95) was used to identify significant differences in endpoints measured in the seven day daphnid tests.

For discussion purpose no observed effect concentrations (NOECs) were used. A NOEC is defined as the highest concentration of an effluent or metal in a toxicity test that does not cause an observable adverse effect.

Biological surveys

In conjunction with the receiving water and effluent toxicity tests of Mount et al. [3], an ecological survey including the quantitative assessment of the periphytic, zooplankton, benthic macroinvertebrate, and fish communities were conducted at each water sampling station. Only the selected results of the ecological survey, germane to determining if the site-specific water quality criteria derived for copper and zinc were protective of aquatic life, will be presented in this paper.

Quality Assurance

Coordination of various studies was completed by the authors. Details of sampling, transfer of samples, storage of samples, specific sampling

sites, dates of collections and measurements to be made on each sample were delineated. We were responsible for all quality assurance related decisions onsite or in the laboratory.

All instruments were calibrated by methods provided by the manufacturers. Methods in the referenced published reports were followed.

RESULTS AND DISCUSSION

Physical and chemical

The physical and chemical measurements (Table 1) made on Naugatuck River water samples and point source effluent samples collected between 23 and 29 August 1983 demonstrate that the river is a multi-effluent complexed system containing the metals of interest (Table 2). Copper and zinc concentrations were relatively low in station 1 water samples when compared to water samples collected downstream which contained elevated concentrations of copper and zinc that were directly attributable to the concentrations in the effluents (Table 2). The impacts of the effluents on water quality are also evident in the hardness, turbidity, total suspended solids, total organic carbon, conductivity, nitrite, nitrate, and ammonia measurements which differed between station 1 and all other stations (Table 1). Data for selected physical and chemical parameters measured on water collected from station 1, 4A, 5, 6, and 7 on 31 August 1983 (Table 3) and from station 1 on 5 September 1983 for use in the copper and zinc addition toxicity tests were within the ranges obtained during the above seven day period for respective sampling stations.

Life cycle - Metal addition tests

Copper toxicity -- Ceriodaphnia survival and young production were not affected by copper added to station 1 water at total copper concentrations ranging from 3 to 12 µg/l when compared to the control (Table 4). Compared to the control, a 62.7% reduction in survival and 97% reduction in the mean number of young per original female daphnid exposed occurred at the 32 µg/l total copper exposure. None survived to produce young at the 91 µg/l total copper exposure. A 48 hr LC50 of 44 µg/l total copper with a 95% confidence interval of 34-57 µg/l were calculated from the data.

Zinc toxicity -- Ceriodaphnia survival and young production were not affected at total zinc concentrations ranging from 7 to 102 µg/l total zinc in station 1 water when compared to the controls (Table 5). Four days after the test was started, it was evident that the 102 µg/l zinc exposure was not toxic. At this time two additional treatments of 273 µg/l total zinc were added. All of the test animals died within 48 hrs at this treatment. These data were used to calculate a 48 hr LC50 of 163 µg/l for total zinc.

Acute toxicity tests

The estimated total and dissolved copper 48 hr LC50s for Ceriodaphnia determined in reference and station 1 water were essentially the same (Table 6). These values are indicative of a similar copper biological availability and/or toxicity in each water sample. Decreased biological availability and/or toxicity of copper was evident in the LC50 values for station 4A, 5, 6 and 7. These downstream total and dissolved copper LC50 values ranged from 3.2 to 7.1 and 2.6 to 4.6 times, respectively, greater than the station 1 LC50 value. Such increases in total and dissolved copper LC50 values are not surprising, since the binding capacity for copper of waters containing treated domestic sewage are generally much greater than that of similar waters containing less or no treated sewage [9].

The results of the concurrent acute toxicity tests in which the laboratory reared and field collected daphnid species were both exposed in replicate copper concentrations in reference water and water collected from stations 4A, 5, 6, and 7 on 31 August 1983 indicate that they were of near equal sensitivity to copper (Table 6). The total and dissolved copper LC50 values for the laboratory reared daphnids differed from the field collected daphnid LC50 values at most by a factor of 1.3.

Although the fathead minnow larvae were less sensitive to copper than the daphnids, a trend of decreased copper biological availability and/or toxicity between station 1 and stations 4A, 5, 6, and 7 was also evident in the fathead minnow 96-hr total and dissolved LC50 data (Table 7). The station 1 total and dissolved copper LC50 values, however, were 3.1 to 3.3 times greater than the reference water LC50 and indicative of a difference in biological availability and/or toxicity between these test waters.

The LC50s and EC50s for fathead minnows in terms of total, dissolved and free copper for the different site waters (Table 7) are based on measurements of water samples collected at the beginning of each test. In going from the Lake Superior water to the relatively clean station 1 water to the waters carrying increasing effluent loads (stations 4A, 5, 6, 7), the LC50 values in terms of not only total and dissolved copper, but also the free copper, generally increase. The proportional increases in the free copper LC50 values are often also substantial. That indicates that the decrease in copper toxicity downstream is not just due to an increase in the binding capacity of the water due to municipal sewage effluents, since if that was the case, the increases in the free copper LC50 values (if any) would be much smaller. Therefore, there appear to be factors introduced downstream which cannot only bind copper, but reduce the toxicity of the non-bound bioavailable fraction. One of those factors may be hardness since hardness values increase monotonically (36 to 90 µg/l) in going downstream from station 1 to station 7 (Table 7). However, with this increase in hardness, only a 2.4 fold decrease in toxicity would have been expected at station 7 based on the hardness correction factor used in the National Criteria Document for Copper [10].

In contrast to the LC50 values, the EC50 values in terms of total, dissolved, and free copper (Table 7) remain relatively constant in going from the station 1 water to the various waters downstream (stations 5, 6, 7). The only major anomaly is the free copper EC50 for the station 4A water. The free copper EC50 value in station 4A water is lower than in station 1 water. This anomaly may have been at least partially due to possible errors in the initial determinations of the free copper activities in station 4A test waters. If the free copper LC50 value for the 4A test water is based on measurements at the end of the test, it is similar to that determined in station 1 water. The relative constancy of EC50 values compared to the increasing LC50 values going downstream may possibly be due to some kinetics effect that decreases the bioavailable fraction during the duration of the 96 hour tests.

The above postulate is based on the following reasoning. During a 96 hour test, the exposure of an organism to a given chemical at the gill membrane is given by

$$\text{Total Exposure} = \int_{0}^{96\ \text{hr}} Q(t) C_{BAF}(t) dt$$

where

$Q(t)$ = flow of water across the gills as a function of time

$C_{BAF}(t)$ = concentration of the bioavailable fraction of the chemical

It should require less total exposure for an organism to develop and to exhibit mobility impairment than for it to be killed. If the kinetics of binding are slow enough such that the exposure of the organism is sufficient to cause mobility impairment before a substantial reduction in the bioavailable fraction occurs, but fast enough to lower the bioavailable fraction of the chemical substantially before lethal amounts of exposure

occur, the EC50s should be less dependent upon the binding capacity of the water than the LC50s.

The postulate is supported by free copper determinations in waters bracketing the LC50 point after the termination of the tests. The free copper concentration should be at least somewhat proportional to the bioavailable fraction. The free copper concentration in station 5 and station 6 test waters which bracket the LC50 point decreased by close to 50% and 25%, respectively, by the end of the test. Those are the two waters which show the largest increase in LC50 values from those upstream. The lower of the two free copper concentrations in station 7 water which bracketed the LC50 decreased by 48% while the higher concentration increased by 24%. The decrease in the lower concentration supports the postulate while the increase in the higher concentration, although unexplained, neither supports nor detracts from the postulate.

Substantial periphyton growths were observed in all of the fathead minnow test chambers at the end of the tests in stations 4A, 5, 6, and 7 test waters. Periphyton growth can cause pH and dissolved oxygen fluctuations during the tests which can affect the biological available copper and total exposure of the organisms. Evidence of such fluctuations can be seen in the final pH and dissolved oxygen measurements (Table 8). These factors can affect not only acute values in terms of the free copper, but also acute values in terms of the total and dissolved copper. However, during the first 24 hr of the fathead minnow exposures in station 1, 4A, 5, and 7 waters, when periphyton effects on water quality are thought to have been minimal, impaired mobility and/or mortality was observed resulting in 24 hr EC50 values identical to the 96 hr EC50 values presented in Table 7. For station 6, impaired mobility was not seen at 24 hr but was observed at 48 hr

resulting in EC50 values identical to that calculated for 96 hr. In reference water tests, no impaired mobility of fathead minnows was observed after 48 hr of exposure resulting in identical LC50 and EC50 values. The total, dissolved, and free copper fathead minnow 48 hr LC50 and EC50 values and 95% confidence intervals in parentheses were 94 (59-148), 91 (56-147), and 3.5 (2-6) µg/l, respectively. From the above results it was concluded that the EC50 values were more useful than the LC50 values for calculating water effect ratios reflective of the differences in the biological availability and/or toxicity between river water samples.

The total and dissolved zinc 48 hr LC50 values for Ceriodaphnia determined in reference water and station 1 water differed by a factor of only 1.1 indicating that zinc was of similar biological availability and/or toxicity in each water (Table 9). Larger downstream LC50 values indicate that zinc was less biologically available and/or toxic in the downstream test waters.

The fathead minnow 96 hr zinc LC50 and EC50 values (Table 10) indicate that zinc added to station 1 water was slightly more biologically available and/or toxic than in reference water. Compared to station 1 LC50 values, downstream values were greater at most by a factor of 3.5. These factors reflect a general trend of decreased zinc biological availability and/or toxicity at the downstream stations similar to that observed for the daphnids. The stepwise increase in fathead minnow toxicity values from upstream to downstream appear to reflect increases in water hardness, a factor known to affect zinc toxicity. For example with the 36 to 90 µg/l water hardness increase (Table 3) from station 1 to station 7, a two fold increase in toxicity values occurred and would have been expected based on the hardness correction factor for zinc toxicity used in the national

criteria document for zinc [11]. This zinc-hardness relationship indicates that the domestic and industrial effluents within this river reach from station 1 to station 7 had little or no effect on zinc bioavailability and/or toxicity other than their contribution to water hardness.

The total copper LC50 values derived from the Ceriodaphnia 48 hr acute toxicity tests in which the organisms were not fed differed by two fold from the 48 hr LC50 values derived from the seven day metal addition tests in which the organisms were fed. The addition of yeast may have contributed to a reduction in the bioavailability and/or toxicity of copper. No such difference was apparent between zinc LC50 values determined in station 1 water under fed and unfed conditions.

Receiving water toxicity

Generally, the total copper LC50 values for Ceriodaphnia are reflective of the presence or absence of receiving water toxicity to this species (Figures 2A and 2B). For example, the total copper LC50 values were not exceeded by the mean of the total copper concentrations measured in the river water samples from stations 1, 4A, and 5 which were not toxic whereas the LC50 values were essentially the same as the mean of the total copper concentration measured for the water samples from station 6 and 7 which were intermittently toxic in the mass balance toxicity tests of Mount et al. [3]. Such a relationship was not evident for the total zinc LC50 values because the mean zinc concentration of the samples were all lower than the zinc LC50 values (Figure 3).

Reduced biological availability and/or toxicity of copper in downstream Naugatuck River water can be inferred from the results of the seven day

copper addition (Table 4) and mass balance toxicity test [3] conducted with Ceriodaphnia using water collected on 26 August 1983. Except for water quality characteristics these tests were conducted under similar conditions. For stations 2 through 5 (Figures 4A and 4B) water samples, total copper concentrations ranged from 9 to 21 µg/l and survival and young production were similar to that obtained in the controls in the metal addition tests. For the station 6 water sample total copper concentration was 97 µg/l survival and young production were slightly reduced when compared to that in the upstream water samples. At most, these reductions in survival and reproduction are indicative of chronic toxicity. In the copper addition test performed in station 1 water (Table 4), reduction in survival and young production indicative of chronic toxicity were observed at the 32 µg/l total copper concentration. The different total copper concentrations associated with these survival and reproduction endpoints are thought to be reflective of differences in copper biological availability and/or toxicity caused by differences in the physical and chemical characteristics between station 6 and station 1 waters.

The differences in toxicity (reflected as effects on Ceriodaphnia survival and young production) in the mass balance toxicity tests [3] between downstream water samples collected on 26 August 1983 appear to be correlated with relative increases and decreases in copper concentration (Figures 4A and 4B). For example, station 6 water contained 97 µg/l total copper and was, at most, chronically toxic to Ceriodaphnia. Station 7 water contained 146 µg/l total copper and was acutely toxic. Station 8 water contained 201 µg/l total copper and was more acutely toxic than station 7 water. Station 9 water

contained less total copper (114 mg/l) than station 8 and was chronically toxic. Station 10 water contained even less copper (67 µg/l) and was not toxic.

Total copper concentrations associated with toxicity endpoints measured in the Ceriodaphnia mass balance toxicity tests [3] indicate that the bioavailability and/or toxicity of copper fluctuated through time. For example, for station 6, total copper concentrations of the seven samples tested ranged from 77 to 108 µg/l. The water sample containing the highest total copper concentration (108 µg/l) was collected on the first sampling day and correlates with the most severe toxic effects observed in the tests of water samples from this station (Figures 5A and 5B). In this water 95% of the test organisms survived for 48 hr but all were dead within 96 hr of exposure and no young were produced. Less severe effects were evident in the tests using water collected from this station on the second, third, and fourth day of the sampling period in which total copper concentrations ranged from 82-100 µg/l. Daphnid survival after 48 hr of exposure in these waters ranged from 70 to 80% and toxicity was reflected by low young production when compared to young production in waters collected on the fifth, sixth, and seventh day of the sampling period. Daphnid survival and young production in these latter collected water samples were not affected at total copper concentrations ranging from 77-99 µg/l. The differences in toxic response observed for the daphnids between these two groups of station 6 water samples, with practically identical ranges of total copper concentrations, indicate that the biological availability and/or toxicity of the copper also differed. Such differences are also evident in the station 7 data. The total copper concentration in station 7 water samples ranged from 87 to 177 µg/l. Water samples collected on the first and second day of sampling

(Figures 6A and 6B) were essentially identical in total copper concentration (124 and 122 µg/l), however, the water collected on the first day was not acutely toxic, 80% of the daphnids survived for 48 hr and survival decreased to 50% by the end of the test resulting in relatively low young production whereas effects on daphnid survival and young production were more severe in the water collected on the second day. This water was acutely toxic and no young were produced. Test waters collected on the next three sampling days were also acutely toxic. For these waters total copper concentrations ranged from 122 to 166 µg/l. No daphnids exposed to these samples survived to produce young. The water collected on the sixth sampling day contained 68 µg/l total copper. Copper concentration was affected by dilution from runoff from heavy rain-fall in the upstream watershed. In this water sample, 70 percent of the organisms were alive after 48 hr of exposure and were still alive at the end of the test. Young production was relatively high when compared to that produced in previously collected water samples. The water collected on the seventh sampling day contained 157 µg/l total copper but was not toxic. Ninety percent of the daphnids were alive after 48 hr exposure, 80% survived to the end of the test and produced young. In this water the total copper concentration was higher than in the toxic water samples collected throughout the first four days of the sampling period. The lack of toxic response is indicative of lessened copper biological availability and/or toxicity and correlates with a marked increase in turbidity. Turbidity measurements ranged from 2.3 to 3.2 N.T.U. for the preceding six water samples and was 10.9 N.T.U. on the seventh day.

The severity of toxic effects on Ceriodaphnia survival and young production measured in the mass balance tests [3] of station 6, 7, 8, and 9

water samples are correlated with mean total copper concentrations. For station 5, mean survival was 93% after 48 hr of exposure and the grand mean number of young produced by the end of the seven day test period was 16.2. The marked increase from 16 to 93 µg/l in mean total copper concentration between station 5 and 6 water samples correlates with a 25% reduction in the mean number of young produced compared to station 5 (Figures 2A and 2B). The sequentially higher mean total copper concentrations of 139 and 185 µg/l for station 7 and 8 water samples correlate with a 55 and 84% reduction in survival and 67 and 99% reduction in young produced, respectively, when compared to station 5. Compared to station 8, the mean total copper concentration decrease to 112 µg/l for station 9 correlates with increases in survival and the number of young produced. For this station, 48 hr survival of the daphnids was similar to that obtained in station 5 water but the mean number of young produced was 62% less.

For station 10, contrasting results were observed. The mean copper concentration for station 10 water (80 µg/l) was less than for station 9 (112 µg/l). The mean number of young produced in station 10 water was greater than in station 9 water as would be expected due to the lower mean copper concentration in station 10 water. However, the mean survival in station 10 water was 26% lower than in station 9 water. Thus with the mean total copper concentration decrease from station 9 to 10, mean survival did not increase as expected. The mortality which lowered the mean survival value occurred on 24 and 25 August 1983 and has been attributed to slug doses of effluent from the Naugatuck STP which also are thought to have killed the fathead minnows in receiving water and effluent toxicity tests [3]. This effluent contained very little copper. The copper concentration of the effluent samples were 11 and 12 µg/l on the above dates.

The total copper concentration of station 1, 4A, 5, 6, and 7 water samples, that were not toxic in the fathead minnow impact toxicity tests of Mount et al. [3], were all less than the total copper EC50 values determined using water collected from the respective sampling stations at a later date (Figure 7A). Station 8 water was toxic to the fish. Toxicity was evident as a marked reduction in survival and growth when compared to that obtained in upstream water samples (Figure 7B). This toxicity correlates with a mean total copper concentration of 185 µg/l and range of 161 to 284 µg/l for the seven water samples used in the test. The high concentration of this range is essentially the same as the 24 hr EC50 of 282 µg/l copper determined in the acute toxicity test in which copper was added to station 7 water. The mean total copper concentration of the station 7 water samples was 139 µg/l and is representative of copper concentration 2 kilometers upstream from station 8.

The toxicity observed at station 8 was associated with the copper added to the system from the Mad River tributary located between stations 7 and 8. Station 9 water samples which were not toxic also contained less copper than the station 8 water samples. The mean total copper concentration for this station was 112 µg/l. Reduction of river copper concentration to this level was associated with the dilution of the river water by the Waterbury STP effluent located between stations 8 and 9. This effluent averaged 38.4% of the river flow after mixing for the period 22-26 August 1983 [3]. It can be inferred from this information that the presence (station 8) or absence (station 7) of Mad River copper in the river system correlates with the presence or absence of toxicity, and also that toxicity and total copper concentrations were reduced by dilution with the Waterbury STP effluent. For station 10 water samples mean total copper concentrations decreased compared

to station 9, however, toxicity occurred (Figure 6A and 6B). This toxicity is thought due to unidentified organic toxicants in the Naugatuck STP effluent which contained only a mean concentration of 11 (N=4) µg/l total copper but was extremely toxic (100% mortality was observed at a 1% effluent concentration) to fathead minnows in the effluent dilution test [3]. Because of this toxic source, metal concentration and toxicity correlation were not attempted at station 10, 11 and 12. The highest total zinc concentration measured in the water samples used in the above fathead minnow impact toxicity tests was 162 µg/l. This concentration was measured in station 8 water and was 5 times less than total zinc LC50 determined in the acute toxicity test in which zinc was added to station 7 water (Figure 3). Overall these data indicate that the toxicity of station 8 water samples (to fathead minnows) was at least partially, if not all, caused by copper.

Effluent toxicity -- The acute (48 hr LC50) and chronic toxicity (NOEC) values determined for Ceriodaphnia in the off-site effluent dilution tests [3], conducted using water samples collected on 26 August 1983, appear to be directly attributable to copper concentration when compared to the results from the copper addition toxicity test in station 1 water. In all of these tests station 1 water collected on this date was used as dilution water. Although the 48 hr LC50 values for Gulf Stream, Great Brook, Steel Brook, station 8, and Thomaston STP water samples ranged from 1.6 to 53 percent effluent (v/v), total copper concentrations calculated from these values differed only slightly and ranged from 27 to 46 µg/l (Table 11). These copper concentrations are essentially the same or similar to the 44 µg/l total copper LC50 value determined from the seven day copper addition test. Likewise, for four of these tests, the NOEC values ranged from 1 to 30 percent effluent (v/v) whereas the total copper concentration calculated from

these values were essentially the same and ranged from 18 to 21 µg/l. These copper concentrations all fall between the no observed effect concentration (NOEC) and the lowest effect concentration range of 12 to 32 µg/l determined in the copper addition test (Table 4). For two tests NOEC of 6 µg/l were calculated and the lowest effect copper concentrations exceeded the copper addition NOEC of 12 µg/l. The total zinc concentrations calculated from the percent effluent LC50 and NOEC values ranged from 8 to 69 µg/l (Table 11). These zinc concentrations are all less than the NOEC of 102 µg/l determined in the zinc addition test (Table 5). These relatively low concentrations of zinc indicate that zinc was not contributing to the acute and chronic toxicity endpoints measured in the effluent dilution tests, however, zinc and other detected metals (Table 12) and unknown components of the water samples tested may have contributed to toxic effects observed at the higher effluent concentrations. Overall, these results indicate that the toxic response of the daphnids observed at the lowest effect effluent concentrations were partially due to copper if not caused by copper.

Copper concentrations associated with the daphnid effluent NOEC(s) of water samples collected over the 7 day sampling period were highly variable (Table 13). For example, the total copper concentration associated with the Thomaston STP effluent NOEC(s) ranged from 4 to 143 µg/l. Where effluent copper NOEC(s) were markedly lower than the 12 µg/l NOEC determined in the copper addition test, the toxicity measured at the lowest effect concentration was probably caused by some other material. Where effluent copper NOEC(s) exceeded this value by a large margin, most of the copper was apparently bound to some other material and not biologically available to the daphnids.

Site-specific water quality criteria

Criteria derivation -- According to the revised national guidelines [12] a water criterion consists of two concentrations: the criterion continuous concentration (CCC) and the criterion maximum concentration (CMC) (the CCC is equivalent to the criterion average concentration of the former national guidelines and the site-specific guidelines). The criterion is stated as: the procedures described in "Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses" indicate that, except possibly where there are multiple discharges, multiple pollutants, or unusually stressful conditions or where a locally important species is very sensitive (1) aquatic organisms should not be affected unacceptably if the four-day average concentration of the material of interest (copper and zinc in this case) does not exceed the CCC more than once every three years on the average and if the one hour average concentration does not exceed the CMC more than once every three years on the average.

National water quality criteria for copper in this study are expressed as total recoverable copper [10]. In this study total acid exchangeable and dissolved copper measurement were made on almost all of the samples used in testing. For comparative purposes total acid exchangeable and total recoverable measurements were also made on the 29 August 1983 water samples from stations 1 through 12 (Table 14). For copper, in all cases, the total acid exchangeable concentration was essentially equal to or less than the total recoverable concentration. These data indicate that total acid exchangeable copper concentrations of the Naugatuck River water equal to or above the criteria concentrations are evidence of the copper criteria being exceeded. National water quality criteria for zinc are expressed as total recoverable

zinc [11]. The total acid exchangeable and total recoverable zinc concentrations measured in each of the above samples were essentially the same. Thus, for zinc criteria evaluation, criteria concentrations equal to or exceeding the total acid exchangeable zinc concentrations are considered evidence of the zinc criteria being exceeded.

In order to derive specific water quality criteria using the indicator species procedure of the site-specific guidelines, the resident species range of sensitivity to the chemical of interest should be similar to that for the species used to establish the national criteria. Species representatives of the genus Daphnia have been identified in the national criteria documents for copper [10] and zinc [11] as the most sensitive to each chemical based on acute toxicity data and among the most sensitive based on chronic toxicity data. Daphnia spp. also inhabit the Naugatuck River [3]. Because of this similarity, it was assumed that the range of sensitivity of the resident species of the Naugatuck River to copper and zinc was similar to that used to establish national criteria for these metals. Furthermore, acute toxicity values for copper determined in reference water with Ceriodaphnia and the resident daphnids Scaphrolebaris (Table 6) indicate they are of near equal sensitivity to the Daphnia spp. cited in the copper national criteria document.

Analysis of the copper LC50 data determined in site (station 1) and laboratory (reference) waters indicate that there was very little if any difference in the biological availability and/or toxicity of copper in these waters. The water effect ratios (site water LC50 value/laboratory water LC50 value) calculated from the total copper LC50 values determined in tests of station 1 water (collected on 31 August 1983) and reference water were 1.0 and 3.4 for Ceriodaphnia and fathead minnows, respectively. For both

species, tests in the site water and reference water were not conducted concurrently and different batches of organisms were used. The larger fathead minnow ratio may be reflective of experimental error and/or differences in copper sensitivity between batches of fish tested. A difference of three fold in total copper LC50 values has been previously observed for different batches obtained from the same breeding stock used in this study [6]. In concurrent tests with each species using station 1 water collected on 5 September 1983 and another reference water sample, similar LC50 values for each species were obtained (Table 15). A 95% confidence interval could not be calculated for the Ceriodaphnia 48 hr LC50 value (18 µg/l) determined in this site water, however, the 24 hr LC50 value was 20 µg/l and its 95% confidence interval of 17-22 µg/l overlaps the reference water 24 and 48 hr LC50 value 95% confidence intervals (which were equal) indicating that these LC50 values are not significantly different. These Ceriodaphnia 48 hr LC50 values for the two dilution waters are essentially the same as those determined in the previous tests (Table 6) indicating that copper biological availability and/or toxicity remained relatively constant between tests. From these data is was concluded that the copper water effect ratio was 1.0.

The Ceriodaphnia and fathead minnow LC50 data obtained from concurrent zinc acute toxicity tests in station 1 water and reference water were similar (Table 9) resulting in water effect ratios of 1.0 and 0.7 respectively. These ratios indicate that there was little if any difference in the biological availability and/or toxicity of zinc in these waters.

According to the indicator species procedure of the site-specific guidelines, if the LC50 values for each indicator species, determined in relatively clean upstream site and laboratory (reference) water, are not

different, then the national CMC concentration is the site-specific CMC. For copper and zinc the site-specific CMC would be based on the water hardness of the reference water used. The mean water hardness of the reference water used in the copper acute toxicity tests was 47 µg/l. At this mean hardness value, the national and site-specific CMC(s) are both 8.1 µg/l copper [10]. For zinc, the water hardness of the reference water used in acute testing was 52 µg/l. At this water hardness the national and site-specific CMC(s) are both 187 µg/l total recoverable zinc [11]. Following the indicator species of the site-specific guidelines, the site-specific CCC concentrations, for both copper and zinc are the same as the national CCCs. These CCC(s) are 6.2 µg/l copper and 47 µg/l total recoverable zinc. The national CCC for zinc (and thus the site-specific CCC) is not hardness dependent and would apply to the whole river system.

Station specific criteria for copper were calculated using the geometric mean of the water effect ratios (Table 16) determined from reference water and stations 4A, 5, 6, and 7 acute toxicity data. For zinc, because there was little or no evidence of effluent effect in the acute toxicity data from the above stations, other than their contributions to water hardness, the station specific zinc CMC(s) are considered the same as the national CMC corrected for hardness. The station specific zinc CCC(s) are also considered the same as the national CCC of 47 µg/l total recoverable zinc. Thus any copper or zinc measurements above the CCC(s) are considered evidence of criteria being exceeded.

Criteria evaluation -- The site-specific and national water quality criteria derived for copper were exceeded at all of the downstream Naugatuck River sampling stations (Table 17). Total acid exchangeable copper concentrations exceeded the CMC(s) in one or more water samples collected at each

station and were in excess of the CCC(s) in all of the water samples. The differences between the criteria and total acid exchangeable copper concentrations for station 2 to 5 water samples were not great, however, more marked differences occurred at the downstream stations (6 through 12).

The site-specific copper criterion based on a water effect ratio of 1 does not take into account the changes in physical and chemical water characteristics caused by the combined input to the Naugatuck River from the tributaries, industries, and STPs, however, station specific criteria for stations 4A, 5, 6, and 7 take these factors into account and are presented in Table 17. The station specific copper criterion for stations 4A and 5 were not exceeded whereas the station specific criterion for stations 6 and 7 were both markedly exceeded.

The site-specific, station specific, and National Criteria derived for zinc were not exceeded at Naugatuck River sampling station 1 through 5. The total acid exchangeable zinc concentration of the four water samples analyzed for each station were less than the CCC of 47 µg/l zinc. This zinc CCC was exceeded by the mean total acid exchangeable zinc concentrations calculated for all of the remaining downstream sampling stations (6 through 12).

The results of the ecological survey [3] indicate that where the station specific copper criteria were exceeded (station 6 and 7), the aquatic community was markedly impacted. The total number and abundance of fish species captured in the upstream sampling stations (Figure 8) were indicative of an abundant and diverse fish community whereas those captured at station 6 and below were indicative of a severely stressed community. The difference in the number and abundance of species between upstream stations are thought to be primarily caused by habitat differences between stations. Influences of the point source effluents were thought to be minimal. Periphyton

community samples from stations 1 through 5 were generally highly diverse when examined using a diversity index and indicative of good water quality within this section of the river (Figure 9), however minor pollution effects were evident in data for stations 2, 3, and 4. Periphyton in the downstream stations (6 through 12) were of low to moderate diversity and indicative of poor to moderate water quality. Macroinvertebrate data were examined using the diversity index and a community loss index. Direct effects on the benthos were attributed to the discharges from the Gulf Stream and Mad River tributaries, the Torrington and Naugatuck STP (POTW) and the Thomaston dam. Direct effects were not as apparent in the number of taxa and density data (Figure 10). The data were interpreted as reflective of the presence of the healthiest benthic community between station 1 through 5 and a lower quality community from station 6 through 8 and the poorest quality community between stations 9 through 12. The downstream trend of decreasing health of the benthos was attributed to a combination of cumulative industrial wastes and habitat differences related to increased flow. The density of the predominant tricopterans in the samples (Figure 11) were reflective of a marked difference in the density between the upstream (1 through 5) and downstream stations (6 through 12). Zooplankton data were thought to be more reflective of the presence of impoundments masking the detection of any STP or tributary effects on abundance and composition.

CONCLUSIONS

From the results of this study, it was concluded that the national and site specific water quality criteria derived for copper in the Naugatuck River would be protective of the rivers aquatic life under low flow conditions. This conclusion is based on the observation that a relatively healthy aquatic community existed where these criteria were exceeded slightly (stations 2 through 5). This was thought to be due to presence of effluent copper which was complexed and in a non-toxic form. Furthermore, examination of monthly water quality monitoring data [11] from October 1981 to August 1983 indicates that copper concentrations continually exceeded the national and site-specific copper criteria at station 5. Over this period of time the reported total recoverable copper concentrations averaged 17 $\pm$ 1 standard deviation of 6.4 and ranged from 8 to 36 µg/l.

Station specific criteria for copper (site within a site criteria) for stations 4A and 5 indicate that the site-specific and national criteria are over-protective at these stations, however, whether or not the station specific criteria are protective could not be discerned from the available data. The only conclusion made is that where the station specific criteria were exceeded (stations 6 and 7), a markedly pollution impaired aquatic community was evident.

It could not be determined whether or not the site-specific, station specific, and national criteria for zinc were protective of the Naugatuck River biota because these criteria were not exceeded in the upstream samples (stations 1 through 5) where a relatively healthy aquatic community was found. However, an impaired aquatic community was observed where the criteria were exceeded (stations 6 and below).

This study demonstrates that numerical water quality criteria for copper, if incorporated in water quality standards or National Pollution Discharge Elimination System permits, could be used to protect Naugatuck River biota from copper toxicity. However, in some of the effluent dilution tests where copper was a relatively low concentration and expected to be non-toxic, effluent toxicity was observed. This toxicity may have been due to combinations of known and unidentified chemicals for which criteria are lacking. In these cases, alternate standards based on effluent toxicity limits such as recommended by the U.S. EPA [12] would be necessary to protect the well-being of the river biota.

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Table 1. Water chemistry data for water samples collected between 23 and 29 August 1983 from the Naugatuck River, tributaries, and STP effluents.

Water Sample	pH Range[a]	Hardness Range[a] (mg/l as $CaCO_3$)	Alkalinity Range[a] (mg/l as $CaCO^3$)	Suspended Solid[b] (mg/l)	Turbidity[a] (N.T.U.)	Conductivity[c] (1/megaohms)	Total Residual Chlorine[b] (mg/l)	Nitrite[b] (NO_2-N) (mg/l)	Nitrate + Nitrite[a] (NO_2+NO_3-N) (ug/l)	Ammonia (NH_3-N)[a] (ug/l)	Total Organic Carbon[b] (mg/l)
1	7.1-7.4	20-44	36-41	<3	0.3-0.9	80-90 (4)	ND[d]	0.01	167-292	12-46	4.2
2	7.5-7.8	28-59	32-46	<3	0.8-1.6	120-190 (3)	ND	0.05	180-734	18-56	4.5
GS1	7.0-7.3	28-62	28-43	<3	0.9-5.6	-	0.06	0.02	485-11,600	62-800	4.7
3	7.2-7.6	33-72	34-60	4	0.8-3.1	160-280 (5)	ND	0.12	527-1,020	29-64	4.2
TRN	6.9-7.3	63-98	76-106		2.2-5.8	-	0.04	0.34	3,050-7,710	6,200-11,200	11.4
4	7.0-7.5	34-71	50-72	9.3	1.7-2.9	230-330 (4)	ND	0.32	800-3,400	115-3,200	6.5
4A	7.4-7.6	31-61	41-46	12	1.7-5.1	280-330 (4)	ND	0.22	2,220-4,010	49-205	6.0
THM	7.4-7.8 (5)	90-1,590	77-240		2.1-8.7	500 (1)	1.46	0.80	1,170-308,000	6,200-44,000	13.0
5	7.3-7.9	41-96	31-42	8	1.2-2.8	280-380 (5)	0.14	0.09	1,370-7,640	140-510	6.7
SB1	7.0-7.2	106-164	46-60		4.4-8.6	315-450 (3)	<0.25[e]	0.76	1,000-28,300	23-63	6.8
6	7.1-7.3	39-82	40-44	12	1.9-5.9	290-335 (4)	ND	0.24	3,960-6,020	44-280	5.5
GB1	6.4-7.2	62-102	28-49		3.0-7.3	-	<0.25[e]	0.30	645-1,960	34-218	5.7
7	7.1-7.5	45-101	41-48	10	2.3-10.9	320-430 (4)	ND	0.19	2,940-5,140	<10-230	7.6
M5	7.1-7.3	77-159	36-46		5.1-7.7	220-280 (5)	<0.03[e]	0.18	645-1,440	150-400	5.3
8	7.1-7.4	46-105	19-41	6	3.5-7.9	320-500 (4)	ND	0.12	302-3,920	56-175	5.5
WTB	7.0-7.2	87-138	66-175		3.2-13.9	360-750 (5)	0.14	0.10	111-500	11,600-18,000	17.3
9	7.1-7.4	47-107	52-82	3.3	2.4-5.4	305-460 (4)	ND	0.36	2,170-2,940	1,650-3,700	8.7
NGT	7.0-7.1	248-481	49-180		4.2-9.7	1,150 (1)	ND	0.10	11-4,410	1,600-27,500	22.1
10	7.3-7.6	58-130	54-75	7.3	2.8-4.6	380-600 (6)	ND	0.38	2,550-3,140	1,500-3,200	9.2
11	7.4-8.2	88-116	48-61	13	2.3-3.1	400-490 (3)	ND	0.54	3,050-3,770	170-1,600	8.0
12	7.1-7.5	67-103	46-52	7	1.5-3.6	420-475 (4)	ND	0.17	3,040-3,880	86-350	7.3

[a] N = 5 to 7

[b] N = 1

[c] N is in parentheses

[d] ND = not detected (<0.005 ug/l)

[e] Interference in measurement observed. Samples diluted 50 times resulting in change in detection limits.

Table 2. Total acid exchangeable and dissolved copper and zinc concentrations for water collected between 23 and 29 August 1983 from the Naugatuck River, STP effluents, and tributaries.

Water Sample	Copper (µg/l)[a]				Zinc (µg/l)[b]			
	Total		Dissolved		Total		Dissolved	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
1	0.9	0.7-1.1	0.8	0.6-1.2	6	3-12	6	1-8
2	11	9-20	9	7-12	17	8-43	7	4-13
GS1	81	58-120	51	29-94	145	85-218	47	37-163
3	12	7-17	8	5-11	21	8-48	13	7-26
TRN	38	21-60	28	20-37	57	42-70	27	20-32
4	19	16-21	13	14-16[c]	29	26-31	11	8-15
4A	13	11-14	9	8-12[c]	18	9-27	14	1-25
TRN	96	60-140[c]	91	30-150[c]	114	64-160	110	27-170
5	16	13-18	11	10-15[c]	23	14-29	11	7-18[d]
SB1	570	472-680	101	85-123	548	368-1,004	103	54-150
6	93	82-100	50	48-50[c]	89	58-146	30	23-44
GB1	1,790	166-3,392	1,350	187-2,237[c]	656	240-970	652	262-922
7	139	122-177	63	46-86	88	73-124	46	38-54
M5	568	240-891	160	70-273	374	301-342	239	130-260
8	185	161-234	83	60-102	162	150-185	127	110-144
WTB	95	38-190[c]	63	25-89[c]	114	103-133	81	47-102
9	112	100-146	55	39-76[c]	92	79-103	46	35-54[d]
NGT	11	9-12[e]	4	1-8[f]	66	33-130[d]	46	18-96[d]
10	80	56-97	39	23-57[c]	55	64-89	35	25-53
11	54	40-76	29	16-36[e]	60	36-77	29	18-37
12	427	187-1,140	168	110-294[f]	112	62-192	81	42-92

[a] Mean and range based on N=7 unless otherwise noted.

[b] Mean and range based on N=4 unless otherwise noted.

[c] N=6

[d] N=3

[e] N=4

[f] N=5

Table 3. Initial chemical and physical data for reference water (Lake Superior source) and Naugatuck River water from station 1, 4A, 5, 6, and 7 collected on 31 August 1983 and used in the acute toxicity tests.

Water Sample	pH	Hardness[a] (mg/l)	Alkalinity[a] (mg/l)	Conductivity (μmhos/cm)	Turbidity (NTU)	Total Residual Chlorine (μg/l)	Nitrite (μg/l)
Reference	7.7	52	55	95	0.1	ND[b]	<2
1	7.5	36	38	90	0.7	ND	<2
4A	7.5	55	42	250	2.2	ND	150
5	7.5	68	40	305	2.3	40	140
6	7.3	82	40	320	3.6	ND	130
7	7.3	90	43	395	3.0	ND	120

[a] As $CaCO_3$

[b] ND = not detected at a limit of 5 μg/l

Table 4. Young production and percentage survival of Ceriodaphnia (N=10) obtained from a seven day copper addition toxicity test in station 1 water collected on 26 August 1983.

Copper Concentration (µg/l)				Young/Female	Standard Deviation	Percentage Survival
Total		Dissolved				
Nominal	Measured[a]	Mean	Range			
100	91	72[b]	71-72	0	-	0[d]
33	32	27[b]	26-27	0.5[d]	1.0	30[d]
11	12	10[c]	9-10	8.1	5.0	80
3	4	5[c]	4-5	13.1	3.7	100
1	3	5[c]	4-5	11.8	5.7	90
0	<3 (Control)	<3[b]	-	14.5	9.1	80[e]

[a] N = 1

[b] N = 2

[c] N = 3

[d] Significantly different than the controls (P = 0.95)

[e] Includes one male

Table 5. Young production and percentage survival of daphnids Ceriodaphnia (N=10) obtained from a seven day zinc test in station 1 water collected on 26 August 1983.

Zinc Concentration (µg/l)						
Total		Dissolved				
Nominal	Measured[a]	Mean	Range	Young/Female	Standard Deviation	Percentage Survival
300	273	292[a]	-	0[d]	-	0[d]
100	102	94[a]	-	10.1	6.1	90
33	47	38[b]	36-40	9.6	5.6	90
11	18	16[c]	14-17	9.5	6.3	90
3.0	7	9[b]	3-14	9.5	6.4	80
1.5	7	5[b]	4-5	14.7	7.4	90[e]
0.0	5 (Control)	11.1[a]	-	14.1	7.3	90[e]

[a] N = 1

[b] N = 2

[c] N = 3

[d] Significantly different than the control (P = 0.95)

[e] Includes one male

Table 6. Forty-eight hour acute toxicity values and 95% confidence intervals in parenthesis for daphnid species exposed to copper added to reference water (Lake Superior source) and to Naugatuck River water from stations 1, 4A, 5, 6, and 7.

Water	Ceriodaphnia Copper LC50 (µg/l)		Scaphrolebaris Copper LC50 (µg/l)	
	Total	Dissolved	Total	Dissolved
Reference	19 (11-31)[a]	18 (10-30)	18 (17-20)	17 (16-19)
1	20 (12-34)[a]	19 (12-33)	- -	- -
4A	64 (51-80)	51 (42-64)	76 (46-126)[a]	61 (33-96)[a]
5	91 (81-101)	78 (69-87)	97 (86-109)	83 (74-94)
6	90 (87-93)	57 (54-61)	121 (110-133)	79 (72-88)
7	142 (77-193)[a]	88 (48-146)[a]	138 (130-146)	85 (81-90)

[a] These are concentrations bracketing the LC50. Confidence intervals could not be calculated.

Table 7. Ninety-six hour toxicity values and 95% confidence intervals in parenthesis for larval fathead minnows Pimephales promelas exposed to copper added to reference water (Lake Superior source) and Naugatuck River water from stations 1, 4A, 5, 6, and 7.

Water	LC50 (µg/l)			EC50 (µg/l)		
	Total Copper	Dissolved Copper	Free Copper	Total Copper	Dissolved Copper	Free Copper
Reference	55 (38-81)	53 (36-78)	1.8 (1.0-3.2)	47 (35-64)	45 (33-61)	1.5 (0.9-2.6)
1	180 (166-195)	163 (151-176)	3.7 (3.1-4.3)	171 (153-196)	156 (139-176)	3.4 (2.6-4.4)
4A	323 (250-417)	240 (188-307)	2.0 (1.1-3.7)	202 (126-320)[a]	153 (89-243)[a]	0.6 (0.1-2.7)[a]
5	511 (439-594)	378 (328-435)	16.1 (12.0-21.7)	229 (143-366)[a]	179 (117-275)[a]	3.3 (1.2-9.0)[a]
6	>998 -	>437	>20.1	265 (181-389)[a]	164 (111-241)[a]	3.5 (1.5-8.2)[a]
7	689 (555-854)	440 (373-518)	14.8 (11.5-18.9)	282 (257-310)	187 (167-209)	3.0 (2.2-4.2)

[a] These are concentrations bracketing the EC50. Confidence intervals could not be calculated.

Table 8. Final water chemistry data for reference water (Lake Superior source) and Naugatuck River water from stations 1, 4A, 5, 6, and 7 collected on 31 August 1983 and used in the acute toxicity tests.

Water	Fathead Minnow				Daphnid			
	Final D.O.[a] (mg/l)		Final pH		Final D.O. (mg/l)		Final pH	
	Control	Range[b]	Control	Range[b]	Control	Range	Control	Range[b]
				Copper Tests				
Reference	7.7	7.4-8.3	7.1	-	8.0	7.9-8.1	-	-
1	7.6	7.4-7.6	7.2	7.0-7.2	7.6	-	-	-
4A	9.4	9.4-12.4	8.5	8.5-9.4	7.5	-	-	-
5	9.9	8.2-11.4	8.2	-	7.6	7.6-8.1	-	-
6	8.7	8.3-13.2	7.7	-	7.8	7.6-8.1	-	-
7	9.2	7.6-11.2	7.9	-	-	7.6-7.8	-	-
				Zinc Tests				
Reference	7.2	7.2-7.3	7.2	-	8.1	8.1-8.2	7.3	7.2-7.3
1	7.5	7.4-7.6	7.4	6.9-7.4	7.9	7.9	7.0	6.8-7.0
4A	12.2	8.4-12.2	9.2	8.4-9.2	-	-	-	-
5	9.1	8.3-9.1	7.2	-	8.2	8.0-8.2	-	-
6	8.4	8.2-8.8	7.6	-	7.8	7.7-7.8	-	-
7	8.8	8.8-9.8	7.8	-	7.3	7.0-7.6	-	-

[a] D.O. = dissolved oxygen

[b] Between two or more treatments.

Table 9. Forty-eight hour acute toxicity values and 95% confidence intervals in parenthesis for Ceriodaphnia exposed to zinc added to reference water (Lake Superior source) and Naugatuck River water from stations 1, 5, 6, and 7.

Water	Zinc LC50 (µg/l)	
	Total	Dissolved
Lake Superior	180 (105-305)[a]	169 (104-308)[a]
1	164[b] (128-217) 149[c] (103-217)	165[b] (126-217) 145[c] (101-210)
5	222 (186-263)	194 (161-233)
6	366 (272-493)	234 (253-443)
7	255 (160-406)	232 (149-361)

[a] These are concentrations bracketing the LC50. Confidence intervals could not be calculated.

[b] The test was started on 1 September 1983 using water collected on 31 August 1983.

[c] The test was started on 3 September 1983 using water collected on 31 August 1983.

Table 10. Ninety-six hour acute toxicity values for larval fathead minnow Pimephales promelas exposed to zinc added to reference water (Lake Superior source) and Naugatuck River water from stations 1, 4A, 5, 6, and 7.

Water	Zinc LC50 (µg/l)		Zinc EC50 (µg/l)	
	Total	Dissolved	Total	Dissolved
Reference	551 (450-677)	550 (451-672)	551 (450-677)	550 (451-672)
1	393 (308-501)	387 (307-489)	188 (107-329)[a]	191 (111-328)[a]
4A	440 (190-1,018)[a]	373 (156-893)[a]	440 (190-1,018)[a]	373 (156-893)[a]
5	556 (475-650)	527 (445-624)	556 (475-650)	527 (445-624)
6	655 (570-752)	576 (532-624)	621 (566-683)	560 (390-1,090)[a]
7	807 (693-969)	742 (614-896)	659 (333-1,070)[a]	601 (361-1,000)[a]

[a] These are concentrations bracketing the toxicity value. Confidence intervals could not be calculated.

Table 11. Ceriodaphnia effluent dilution toxicity test results determined in water samples collected on 26 August 1983. Station 1 water was used as dilution water in all tests.

Water	Acute Toxicity			Chronic Toxicity		
	48 hr LC50 as % Effluent (v/v)	Total[a] Copper (µg/l)	Total[a] Zinc (µg/l)	NOEC as % Effluent (v/v)	Total[a] Copper (µg/l)	Total[a] Zinc (µg/l)
Gulf Stream	53.1 (30-100)[b]	31	64	10	6	12
Torrington STP	>100	>42	>53	10	6	54
Thomaston STP	44.8 (30-100)[b]	27	29	30	20	19
Steel Brooke	6.9 (5.2-9.2)[c]	46	69	3	20	30
Great Brooke	1.6 (1-3)[b]	29	13	1	18	8
Station 8	17.3 (10-30)[b]	34	27	10	21	16

[a] Total copper and zinc concentrations were calculated from concentrations measured in the undiluted effluent samples.

[b] These are concentrations bracketing the LC50s. Confidence interval could not be calculated.

[c] Ninety-five percent confidence interval.

Table 12. Metals detected[a] (µg/l) using ICPAES analysis of water samples from the Naugatuck River, several tributaries, STP effluents, and reference water. Copper and zinc values in parenthesis were determined using atomic absorption spectrophotometry.

Water Sample	Al (5)[b]	Cd (5)	Cr (5)	Cu (6)	Fe (5)	Ni (10)	Zn (5)
				26 August 1983 Samples			
1	ND[c]	ND	ND	ND(1)	267	ND	12(3)
2	ND	ND	ND	ND(9)	173	ND	ND(8)
3	ND	ND	ND	ND(10)	384	ND	15(18)
4	83	ND	ND	20(21)	520	ND	28(27)
4A	228	ND	ND	ND(13)	480	ND	ND(9)
5	64	ND	ND	ND(13)	167	ND	ND(14)
6	98	ND	ND	85(97)	299	ND	152(146)
7	77	ND	6	126(142)	323	19	65(73)
8	528	ND	12	199(201)	490	51	166(158)
9	114	ND	11	108(114)	409	81	92(97)
10	90	ND	7	61(67)	429	57	56(68)
11	61	ND	6	45(50)	470	51	74(78)
12	ND	ND	14	387(356)	244	30	99(98)
GS1	ND	ND	29	52(57)	514	116	112(121)
SB	310	23	39	677(667)	1,435	76	1,000(1,004)
GB1	161	ND	109	1,858(1,778)	579	145	836(811)
M5	94	ND	39	609(608)	734	446	339(342)
TRN	45	ND	ND	35(42)	189	ND	31(43)
THM	109	ND	ND	52(60)	418	ND	51(64)
WTB	ND	ND	11	124(131)	112	262	96(109)
NGT	1,437	ND	ND	ND(9)	69	ND	19(33)
				31 August 1983 Samples			
1	ND	ND	ND	ND(1)	229	ND	ND(3)
4A	58	ND	ND	ND(9)	256	ND	ND -
5	568	ND	ND	ND(21)	496	ND	ND(27)
6	198	ND	ND	66(68)	361	ND	47(47)
7	178	ND	ND	62(73)	384	ND	65(44)
				Lake Superior			
Reference	ND	ND	ND	ND	ND	ND	ND

[a] As, Ba, Hg, Pb, Sb, and Se were not detected at 30, 5, 30, 20, 20, and 30 µg/l.

[b] Detection limit in µg/l.

[c] ND = not detected

Table 13. Range of no observed effect concentrations determined from *Ceriodaphnia* effluent dilution tests conducted using water samples collected between 23 August 1983 and 29 August 1983. Station 1 water was used as dilution water in all tests.

Effluent Samples	No Observed Effect Concentration Range		
	Percent Effluent (v/v)	Total Copper[a] (µg/l)	Total Zinc[a] (µg/l)
Gulf Stream	1-30 (7)[b]	1-26 (7)	1-65 (4)
Torrington STP	3-100 (6)	1-57 (6)	17-70 (4)
Thomaston STP	3-100 (7)	4-143 (7)	2-48 (4)
Steele Brooke	1-3 (6)	6-20 (6)	10-30 (4)
Great Brooke	1-10 (5)	7-18 (5)	6-10 (4)
Mad River	3-30 (2)	8-72 (2)	87 (1)
Station 8	10-30 (7)	18-38 (7)	15-19 (4)

[a] Total copper and zinc concentrations were calculated from concentrations measured in the undiluted samples.

[b] The number of samples represented by the ranges are in parenthesis.

Table 14. Comparison of total acid exchangeable and total recoverable metal concentrations of Naugatuck River samples collected on 29 August 1983.

Station	Copper (µg/l)		Zinc (µg/l)	
	Total Acid Exchangeable	Total Recoverable	Total Acid Exchangeable	Total Recoverable
1	<1.5	<0.5	-	0.7
2	15	15	17	21
3	15	14	24	28
4	19	18	13	16
4A	17	14	15	15
5	15	16	30	26
6	89	102	78	76
7	142	159	123	122
8	221	247	223	207
9	97	117	118	110
10	82	108	111	102
11	42	49	37	44
12	180	208	103	102

Table 15. Acute toxicity values for Ceriodaphnia and larval fathead minnows exposed to copper in reference water (Lake Superior source) and Naugatuck River water collected form station 1 on 5 September 1983.

Water	Ceriodaphnia 48 hr LC50 (µg/l) as total copper	Fathead Minnow 96 hr LC50 (µg/l) as total copper
Reference[a]	17 (15-20)[b]	85 (64-112)[b]
1[c]	18 (13-25)[d]	95 (74-122)[b]

[a] Dissolved copper measurement in two treatments ranged from 100 to 102% of the total copper concentration.

[b] Ninety-five percent confidence interval.

[c] Dissolved copper measurement in two treatments ranged from 95 to 96% of the total copper concentration.

[d] A 95% confidence interval could not be calculated, these are concentrations bracketing the LC50.

Table 16. Water effect ratios for copper (site water LC50/lab water LC50). The daphnid ratios are based on LC50 data and the fish ratios are based on EC50 data.

Station	Ceriodaphnia	Scaphrolebaris	Fathead Minnow	Geometric Mean
4A	3.3	4.2	4.3	3.9
5	4.8	5.4	4.9	5.0
6	4.7	6.7	5.6	5.6
7	7.4	7.7	6.0	7.0

Table 17. Naugatuck River copper concentrations and water quality criteria for copper in µg/l.

River Station	Mean Total Acid Exchangeable Copper	Copper Criterion					
		National		Site-Specific		Station Specific	
		CMC	CCC	CMC	CCC	CMC	CCC
1	1	7.4	5.1	8.7	6.2	8.7	6.2
2	11	9.2	6.5	8.7	6.2	-	-
3	12	10.8	7.5	8.7	6.2	-	-
4	19	11.3	7.8	8.7	6.2	-	-
4A	13	10.3	7.2	8.7	6.2	34	24
5	16	13.1	9.0	8.7	6.2	44	31
6	93	13.3	9.1	8.7	6.2	49	35
7	139	15.0	10.2	8.7	6.2	61	43
8	185	15.7	10.6	8.7	6.2	-	-
9	112	14.9	10.0	8.7	6.2	-	-
10	80	17.6	11.7	8.7	6.2	-	-
11	54	17.6	11.7	8.7	6.2	-	-
12	427	16.7	11.2	8.7	6.2	-	-

[a] N = 7

[b] Based on the mean of the total water hardness value for the seven daily water samples.

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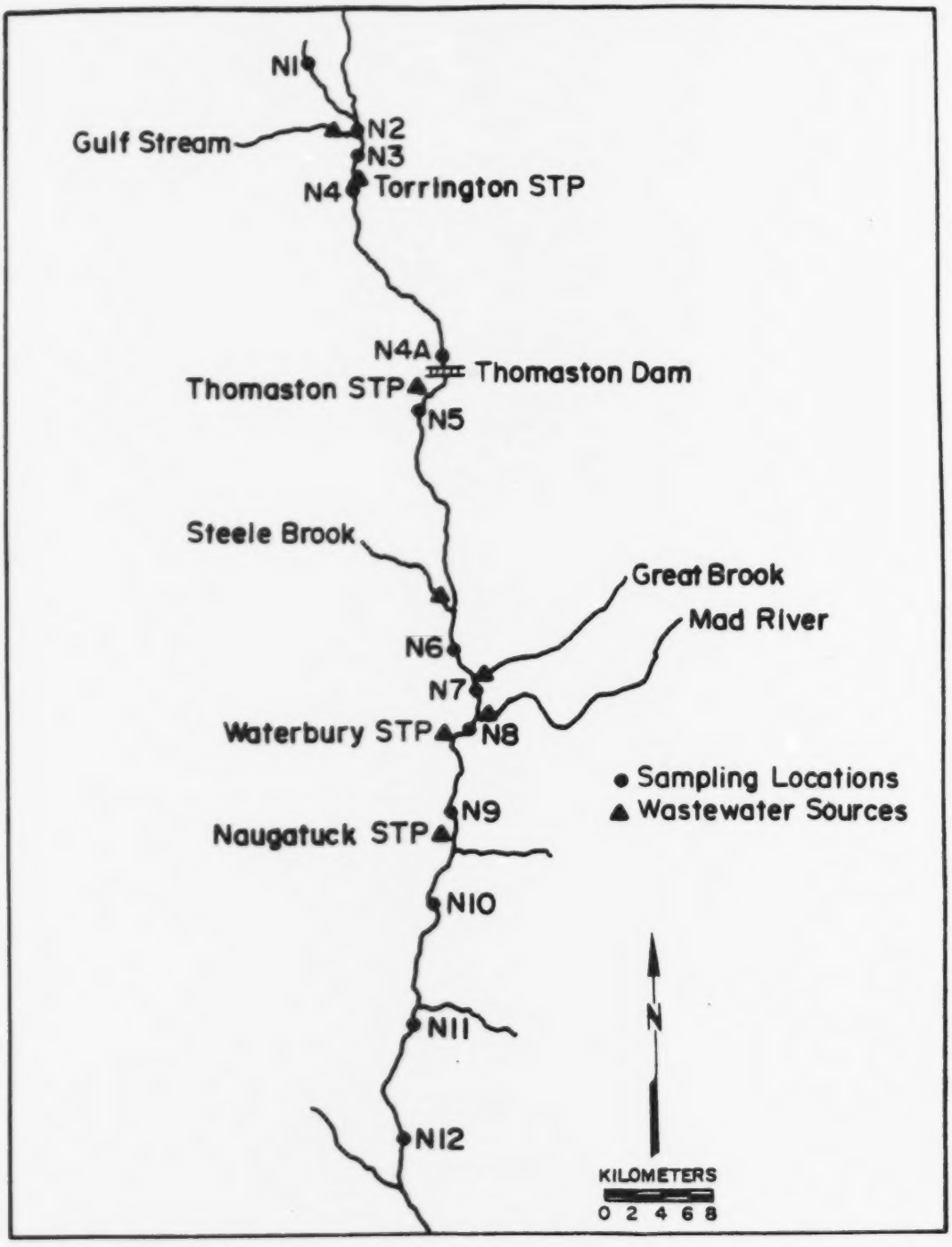

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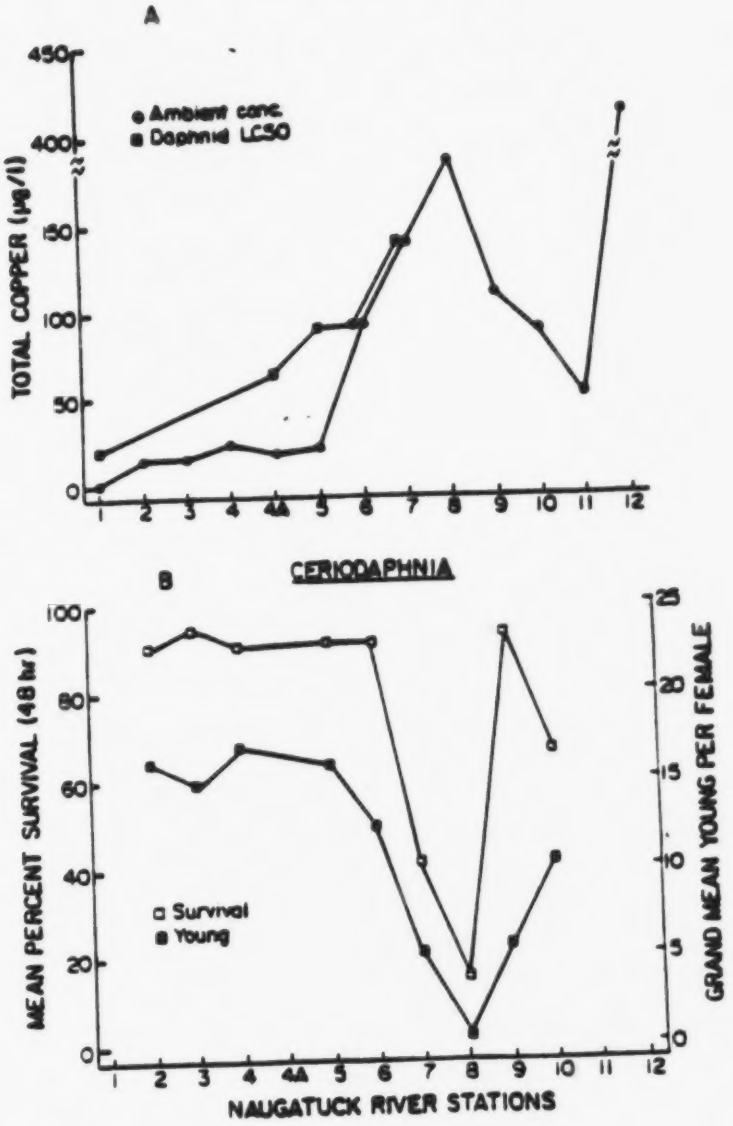

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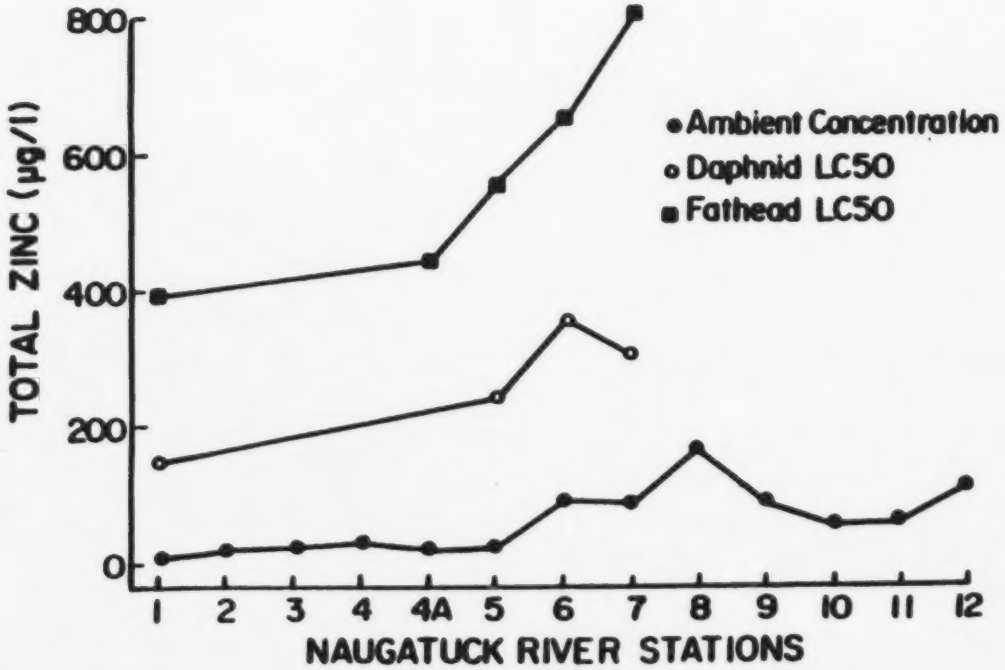

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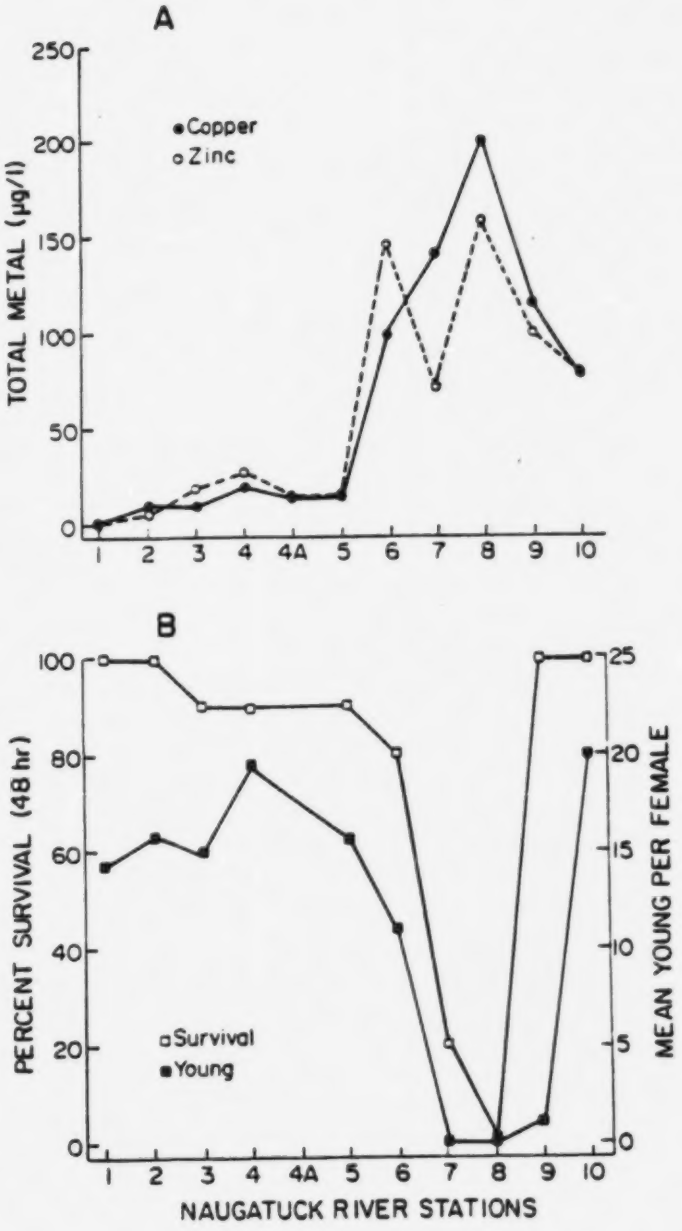

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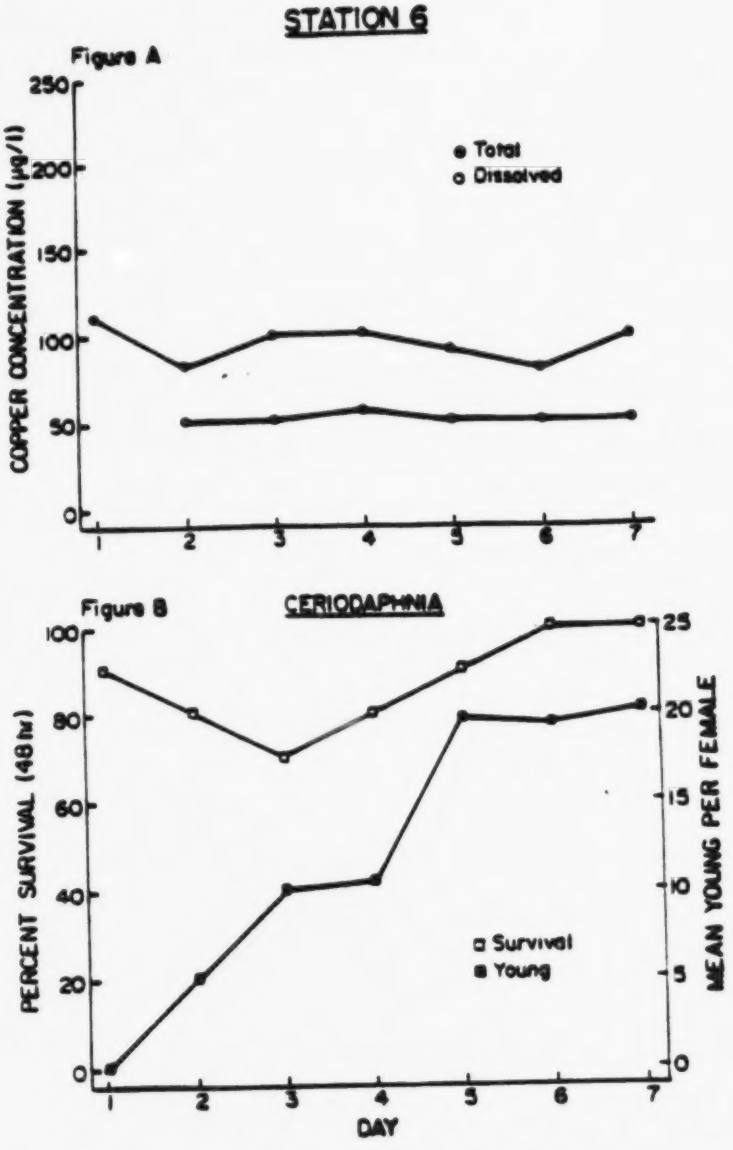

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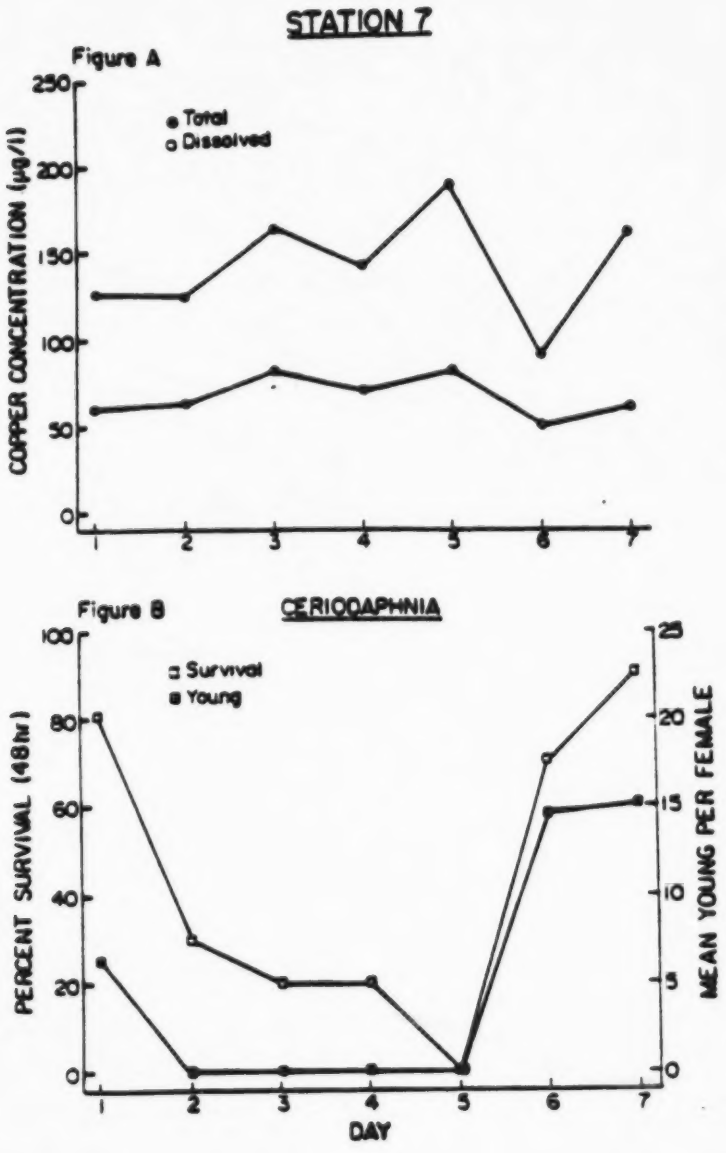

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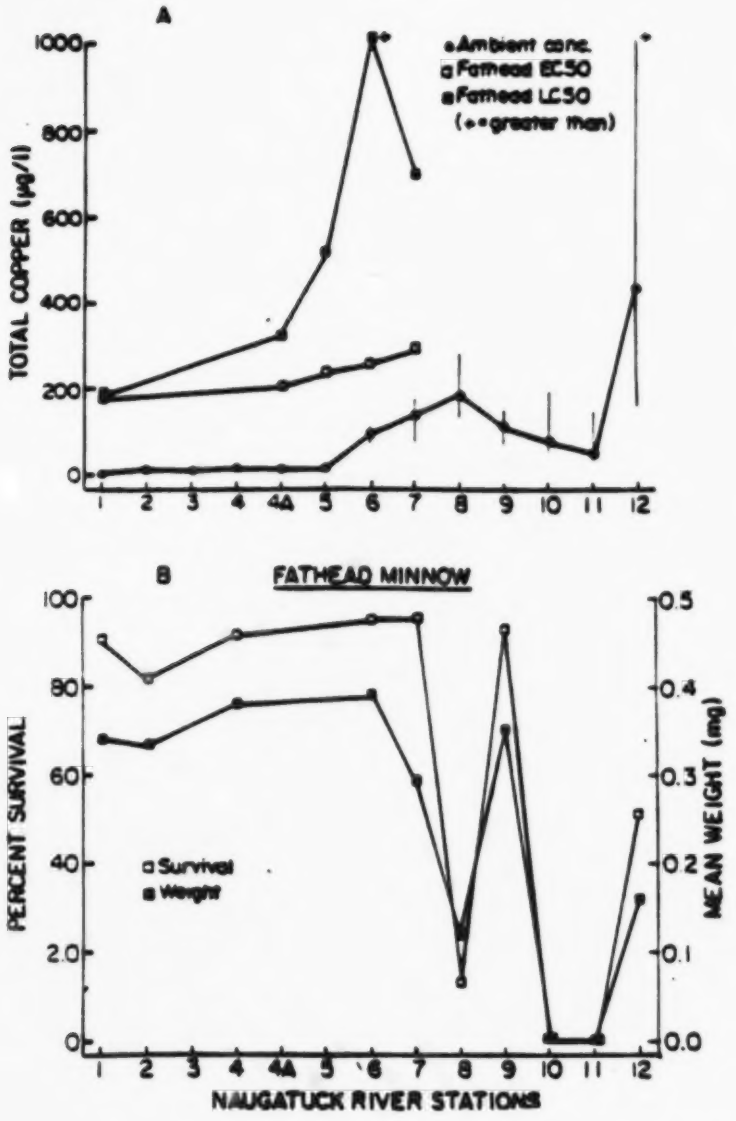

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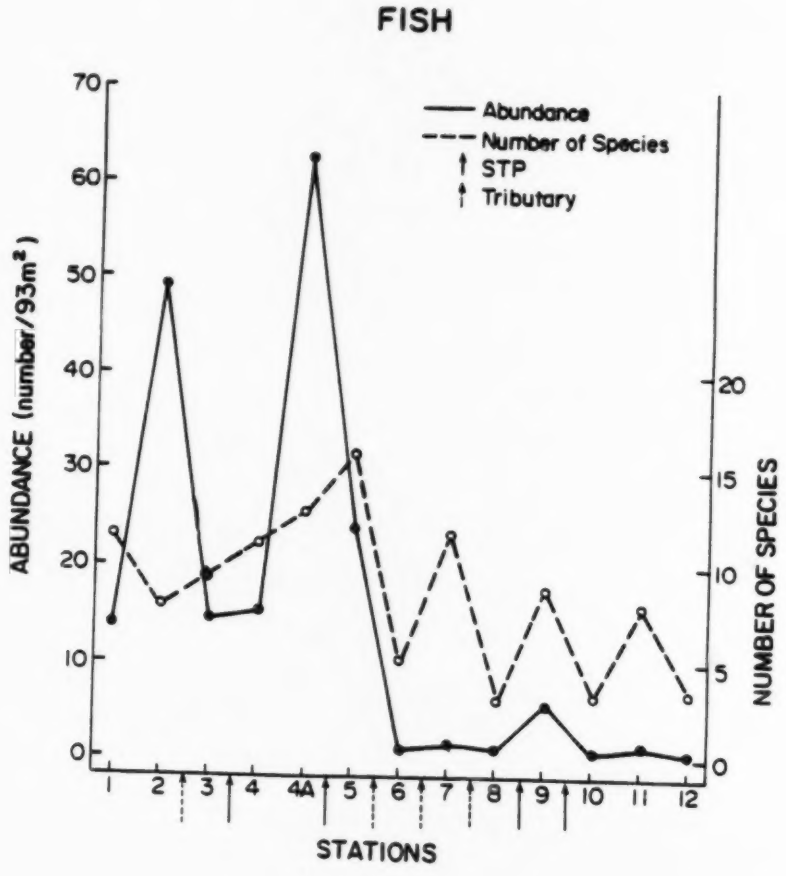

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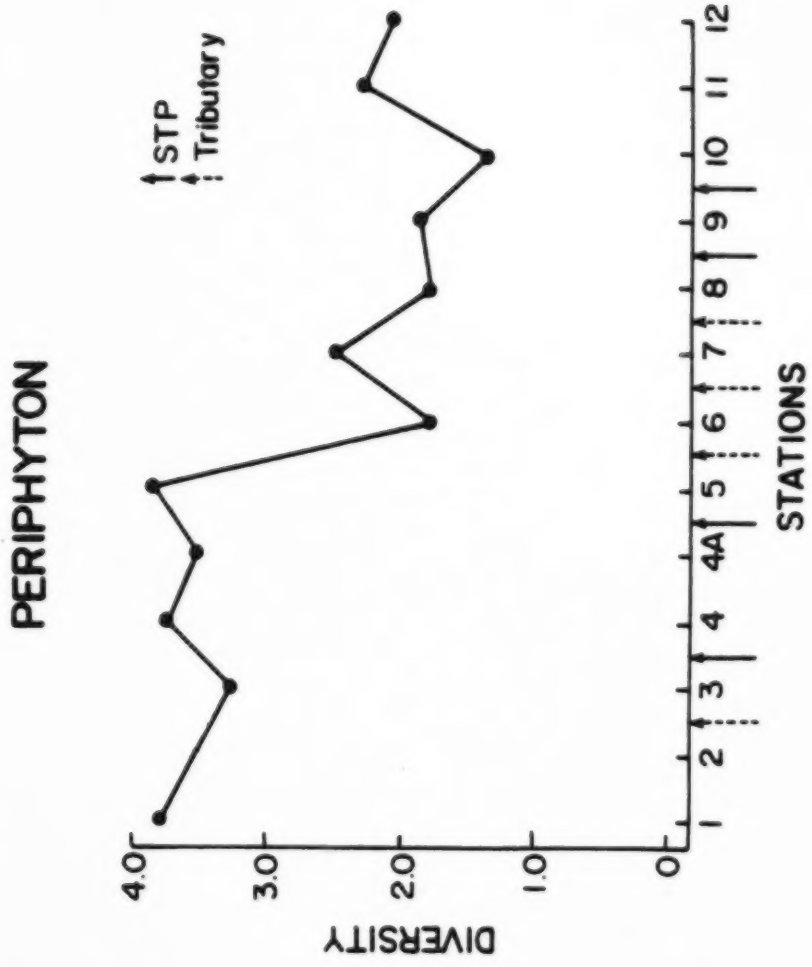

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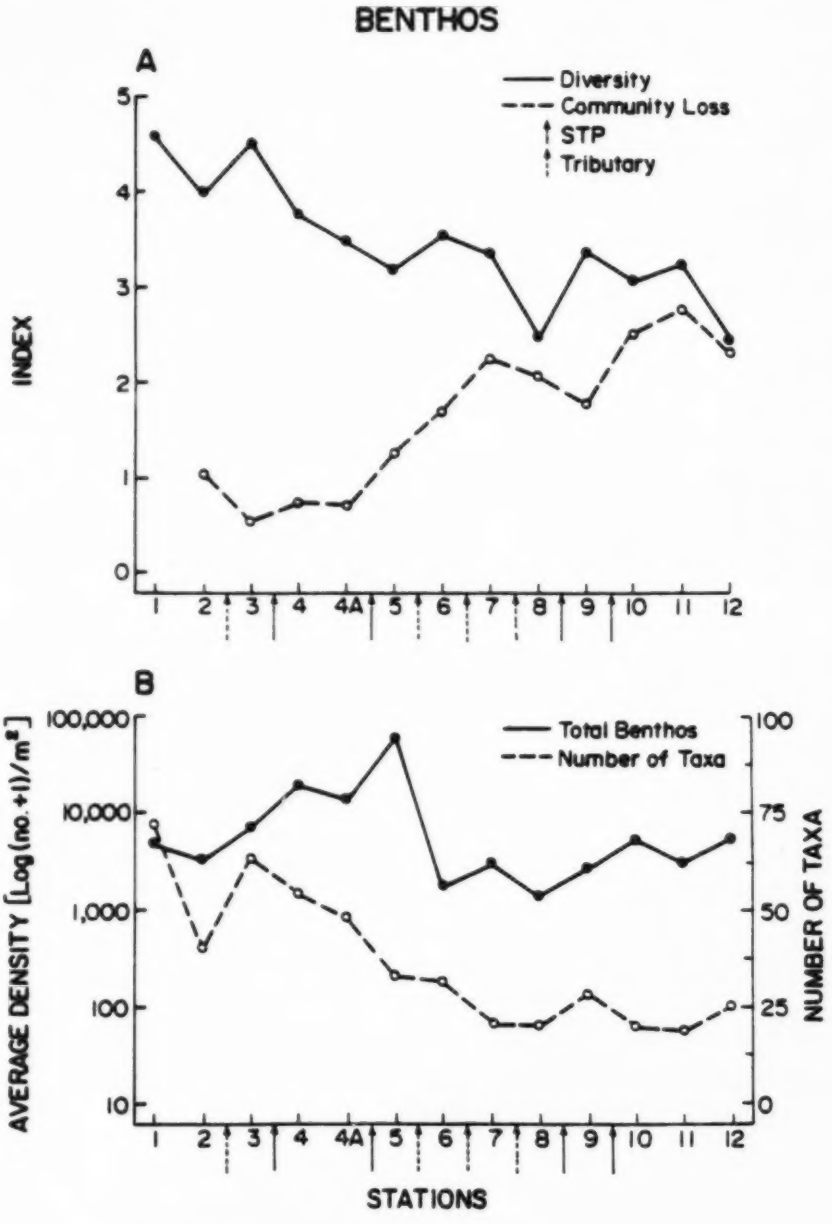

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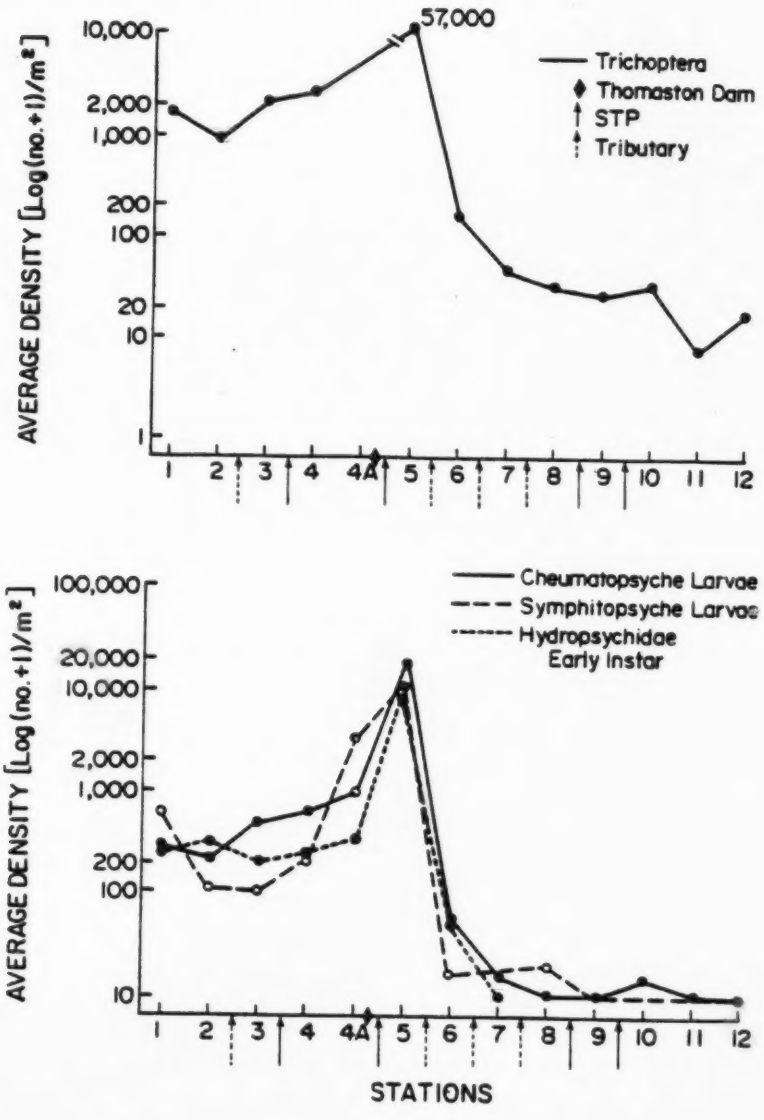

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